Final REPORT ON

CONTRACT NO DA 92-557-FEC-32965

INCLUSIVE DATES 1 March 1965 TO 28 February 1966

48

480196

SUBJECT OF INVESTIGATION

STUDIES ON THE ANTIVIRAL ACTIVITY OF GUANYLHYDRAZONES ESPECIALLY AGAINST ARBO- AND MYXOVIRUSES

RESPONSIBLE INVESTIGATOR

Daizo Nagaki, M.D.

Professor of Microbiology
Kitasato University
Tokyo, Japan

U.S. Army Research & Development Group [redacted] (Far East)
Office of the Chief of Research and Development
United States Army
[redacted]
APO San Francisco 96343

SUMMARY

265 chemical compounds, guanylhydrazones, azlactones, hydrazones, phenols, carbonyl compounds, and miscellaneous ones were tested for in vitro antiviral activity against influenza virus. 104 out of 265 compounds were completely inhibitory by the screening test.

Selected 89 compounds were qualitatively analized for the antiviral and toxic activities. 14 out of 89 compounds were found to be inhibitory with the ratio of 4 or more of non-toxic concentration versus minimal inhibitory concentration.

One compound, serial No.212, synthesis No.178 inhibited HA production with a final concentration of 1.6 γ/cc and was toxic with a final concentration of 25 γ/cc.

STUDIES ON THE ANTIVIRAL ACTIVITY OF GUANYLHYDRAZONES

ESPECIALLY AGAINST ARBO- AND MYXOVIRUSES

Daizo Nagaki, M.D.

Professor of Microbiology
Kitasato University
Tokyo, Japan.

U.S.Army Reseach & Development Group (Far East)
Office of the Chief of Research and Development
United States Army
APO San Francisco 96343

CONTENTS

Introduction..... 1

Materials and Methods..... 1

1. Virus and its titration..... 1

2. Chemical compounds..... 1

3. Hemagglutination test..... 1

4. A) Screening test of antiviral effect of chemical compounds..... 2

B) Determination of antiviral and toxic concentration of chemical compounds..... 2

5. Toxicity test of compounds..... 2

Results..... 3

1. Screening test of antiviral effect of chemical compounds..... 3

2. Determination of antiviral and toxic concentration of chemical compounds..... 3

References..... 4

Table of experimental results..... 5-39

Table I. List of chemical compounds..... 5-28

Table II. Screening test of chemical compounds in chick chorioallantoic membrane.. 29-32

Table III. Result of screening test of 265 compounds..... 33-34

Table IV. Summary of screening test..... 34

Table V. Quantitative analysis of antiviral and toxicity test in membrane..... 35-38

Table VI. Result of antiviral test of 59 compounds..... 39

Table VII. Summary of antiviral test..... 39

INTRODUCTION

It has been reported that poliovirus multiplication was inhibited by guanidine in vitro (Lwoff, 1963). Guanidine derivatives were inhibitory against polio, coxakie, measles, influenza and parainfluenza viruses (Crowther and Melnick, 1961; Tamm and Eggers et al., 1962; Ueda, 1961, 1962; Loddo, 1962).

It was recently reported that amantadine hydrochloride inhibited influenza virus penetration into the host cells (Hoffmann et al., 1965).

This paper reports the screening results of guanylhydrazones, azlactones, hydrazones, phenols and carbonyl compounds with the method of chorio-allantoic membrane culture. All chemical compounds used were synthesized at the Department of Organic Chemistry, Kitasato University College of Hygienic Sciences.

MATERIALS and METHODS

Influenza A virus Adachi strain : Infected allantoic fluid of 80th and 81st egg-passages was ampuled in amount of 1 ml each and stored in a dry-ice box until use. One-tenth ml of serial ten-fold dilution of the stock virus was inoculated into test tube containing a piece chorio-allantoic membrane and egg-shell in Hanks' solution (Iwasaki et al., 1955).

After shaking culture of 4 tubes each dilution for 48 hours at 36°C, hemagglutination titer of each culture fluid was determined by the standard hemagglutination test with chicken red cells. The maximal dilution showing positive hemagglutination in all four tubes was detected.

Serial dilution of the stock virus between this maximal dilution showing positive hemagglutination in all four tubes and the next ten-fold dilution were retested to estimate exact titer of inducing dilution of the stock virus was determined and named 1 MID_{100}. Ten MID_{100} was used as inoculum in the experiments.

2. Chemical compounds

All chemical compounds were synthesized at the Department of Organic Chemistry, Kitasato University college of Hygienic Sciences and dissolved in distilled water or 50 % glycerin. Dissolved compound solutions were autoclaved at 121°C for 20 minutes prior to use. The abbreviations W and G were adopted for distilled water and 50 % glycerin used as solvent, respectively.

3. Hemagglutination test

An equal volume of culture fluid from a group of 4 tubes was mixed and two-fold serial dilutions were made in an amount of 0.4 ml of PBS on plastic-plate. Same amount of 0.5 % chicken red cell suspension was added and allowed to hemagglutinate for 60 minutes at room temperature.

4. A) Screening test of antiviral effect of chemical compounds.

All chemical compounds tested were selected first as inhibitory, partially inhibitory, and non-inhibitory ones by the application of only one concentration as indicated in text.

The content of culture tube was as follows:

chorio-allantoic membrane	Ca. 5 X 10 mm	1	piece
egg-shell	Ca. 5 X 5 mm	1	piece
Hanks' BSS (pH 7.2-7.4)		0.8	ml
virus 10 MID_{100} / 0.1 ml		0.1	ml
Test sample solution		0.1	ml
	Total	1.0	ml.

An equal volume of chemical solution and 10 MID_{100} / 0.1 ml of virus were mixed and allowed to react at room temperature for 30 minutes and two-tenth ml of the mixture was inoculated into four culture tubes for each group containing 0.8 ml of Hanks'BSS, a piece of chorio-allantoic membrane and egg-shell, and appropriate controls were made. The fluid was cultivated in rubber-stoppered test tube (14 X 120 mm) by shaking machine (stroke distance 120 mm : 110 strokes per minute) for 48 hours at 36°C incubating room.

B) Determination of antiviral concentration of chemical compounds.

all chemical compounds could be divided into three groups of inhibitory, partially inhibitory, and non-inhibitory ones by the screening test. Only inhibitory compounds were selected and determined for the minimal inhibitory concentration and non-toxic concentration against virus by the limiting dilution of compounds.

The method of determining minimal concentration of each compound was same as described above and the final concenreation of compound which inhibited completely hemagglutinin production was taken as minimal inhibitory concentration.

5. Toxicity test of compounds.

The test tubes containing chorio-allantoic membrane in 0.9ml of Hanks' BSS was incubated with 0.1 ml of appropriately diluted compound solution for 18 hours at 37°C on shaking machine. The incubated membrane was washed twice with 5 ml of PBS, respectively. The washed membrane was inoculated with 10 MID_{100} / 0.1 ml virus in 1 ml of Hanks' BSS. After 48 hour incubation at 36°C, hemagglutinin titer of culture fluid was determined by the standard hemagglutination test technique. The maximal concentration of each compound which produced hemagglutinin was taken as non-toxic concentration.

RESULTS

1. Screening test of antiviral effect of chemical compounds.

A total of 265 chemical compounds listed in Table 1 were tested for antiviral activity by the application of only one concentration as indicated in text. Compounds were autoclaved prior to use after about 18 hour storage at room temperature. Antiviral activity of each compound was noted in Table II. As shown in Table III, the tested compounds were distributed into such three groups as inhibitory, partially inhibitory, and non-inhibitory ones among each group. The numbers of the inhibitory compounds in each group are as follows :

44 compounds (49.5 %) in group A--guanylhydrazones.
7 compounds (38.8 %) in group B----------carbonyls,
22 compounds (30.1 %) in group D---------azlactones,
17 compounds (53.1 %) in group E----------phenols,
14 compounds (31.8 %) in group F-----miscellaneous.

As summarized in Table IV, a total of 104 compounds (39.2 %) were completely inhibitory, 67 compounds (25.2 %) were partially inhibitory and 94 compounds (35.4 %) were non-inhibitory.

2. Determinations of antiviral and toxic concentrations of chemical compounds.

Quantitative analysis of antiviral and toxic activities of selected compounds showing negative hemagglutinin production by the screening test was performed. Compounds were dissolved and autoclaved prior to use. The procedures used were fully described in Materials and Methods. The minimal concentration of each compound which inhibited completely hemagglutinin production was taken as the minimal inhibitory end point by the inhibition test. The maximal concentration of each compound which produced hemagglutinin was taken as the non-toxic end point by the toxicity test. Two relative concentrations each compounds were listed in Table V. The non-toxic end point and the minimal inhibitory end point were compared for each compounds. The ratio of two final concentrations was listed at the right column in Table V.

As can be seen in Table VI, one compound of group A--guanylhydrazones, three compounds of group D--hydrazones, four compounds of group E--phenols, and six compounds of group F--miscellaneous ones were found to be inhibitory against virus with the effective ratio of 4 or more.

The compound, serial number 212 inhibited completely hemagglutinin production with the final concentration of 1.6 γ/cc and was found to be toxic at the final concentration of 25 γ/cc, therefore the ratio of this compound number 212 was 16.

As summarized in Table VII, a total of 14 compounds were found to be inhibitory with the ratio of 4 or more.

REFERENCES

1) Crowther,D. and Melnick,J.L.: Studies of the inhibitory action of guanidine on poliovirus multiplication in cell culture. Virology 15,65-74,1961.

2) Hoffmann,C.E.,Neumayer,E.M.,Haff,R.F.and Goldsby,R.A.: Mode of action of the antiviral activity of amantadine in tissue culture. J.Bact. 90,623-628,1965.

3) Ishida,N.,Shiratori,T.,Rikimaru,K.,Takayanzgi,K.,Saijo,S., Katsube,J.and Segawa,T.: The effect of certain biguanide derivatives on the multiplication of myxoviruses in tissue culture. J.Antibiotics 15,242-246,1962.

4) Iwasaki,K.,Nishimura,T.,Igarashi,Y.,and Nagaki,D.: Studies on the chemotherapy of influenza virus. 1.Effect of thiothemicarbazone on influenza and Newcastle disease virus multiplication. Kitasato Arch.Exp.Med. 27,31-44,1955.

5) Loddo,B.; Relations between the effect and molecular structure of guanidines. Boll.Soc.Ital.Biol.Sper. 38,488-489,1962.

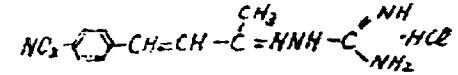
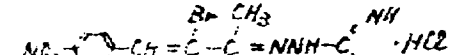
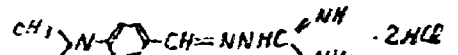
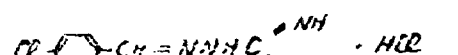
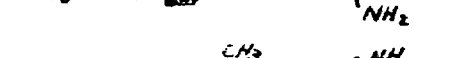
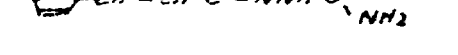
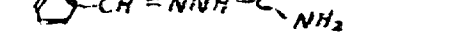
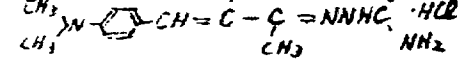
6) Lwoff,A.and Lwoff,M.: Action of guanidine on development of poliovirus. Comp.Rend.,256,5001-5004,1963.

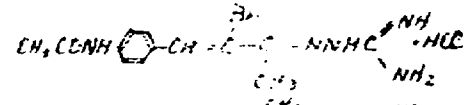
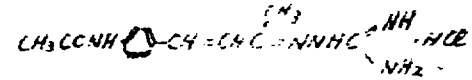
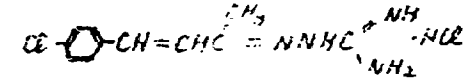
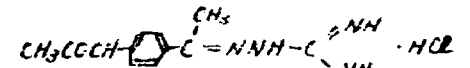
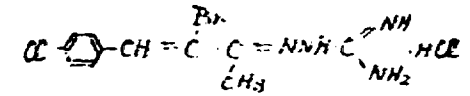
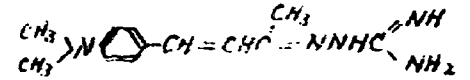
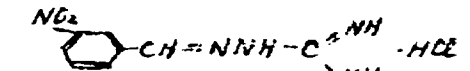
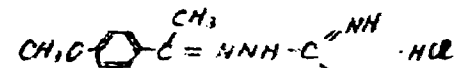
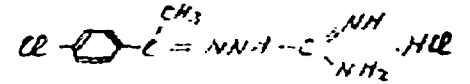
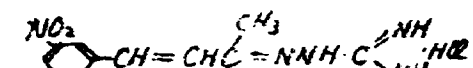
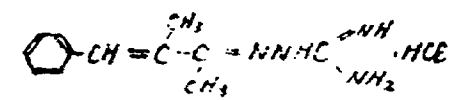
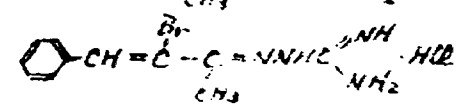
7) Tamm,I. and Eggers,H.J.: Differences in the selective virus inhibitory action of 2-(α-hydroxybenzyl) benzimidazole and guanidine hydrochloride. Virology 18,439-447,1963.

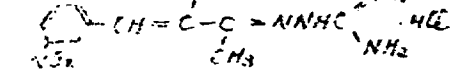
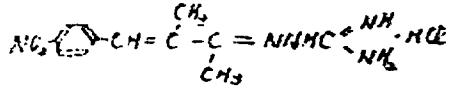
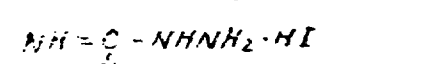
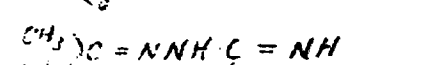
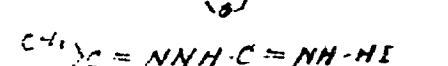
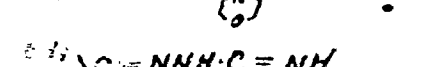
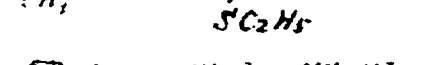
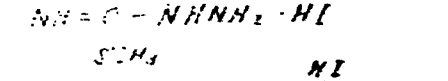
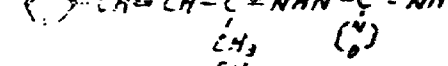
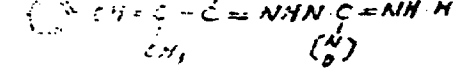
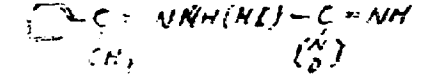
8) Ueda,T.,Toyoshima,S.,Tsuji,T.,Seto,Y.and Nomoto,J.: Antiviral effect of guanidine and its derivatives: 1. The inhibitory effect of guanidine on the multiplication of poliomyelitis virus in tissue culture. Keio J.Med. 10,257-265,1961.

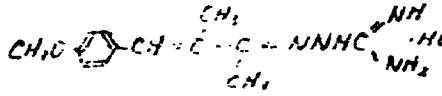
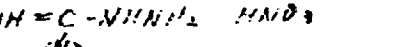
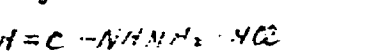
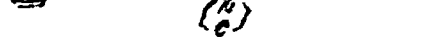
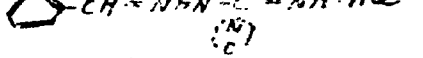
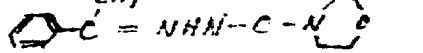
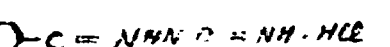
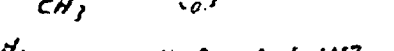
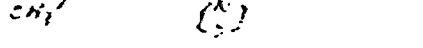
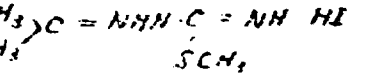
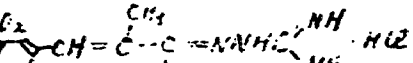
9) Ueda,T.,Toyoshima,S.,Tsuji,T.,Seto,Y.and Nomoto,J.: Antiviral effect of guanidine and its derivatives: 11. The inhibitory effect of guanidine on several virus including measles virus. Antibiot.and Chemothera. 12,330-336,1962.

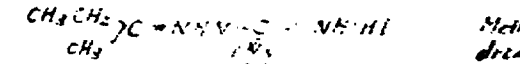
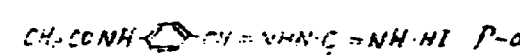
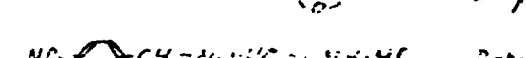
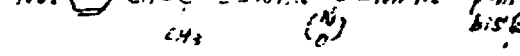
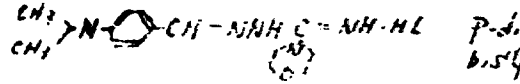
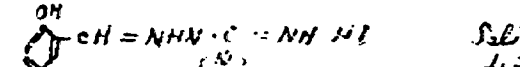
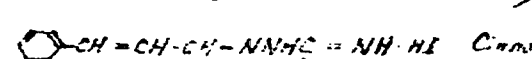
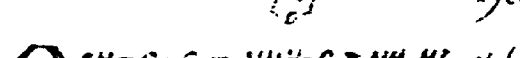
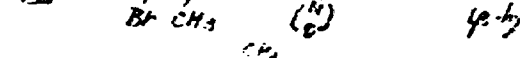
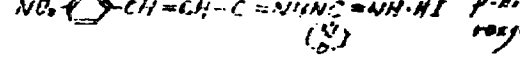
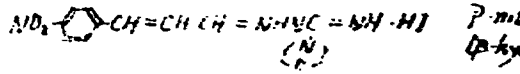
TABLE I List of chemical compounds

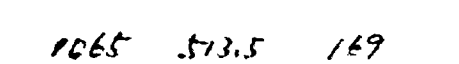
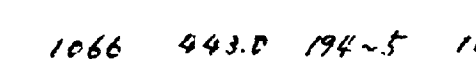
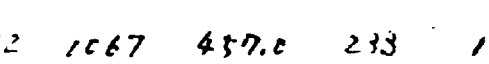
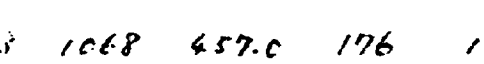
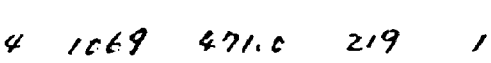
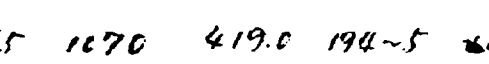
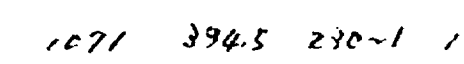
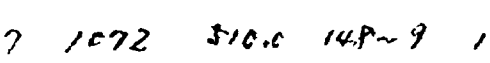
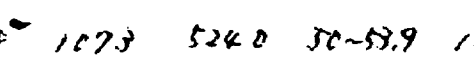
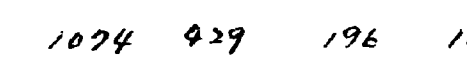
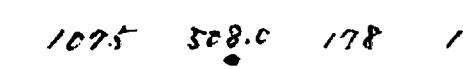
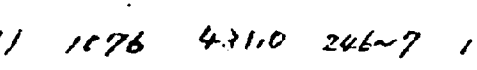
Serial No.	Synthesis No. of compound	Molecular weight	Melting point (°C)	Concentration of Original solution (γ/ml)	Solvent	Chemical structure	Chemical name
	; Group A guanylhydrazone						
1	115	283.5	270	1000	G		P-nitro benzalacetone guanylhydrazone hydrochloride
2	117	362.4	247	1000	G		α-brome-P-nitrobenzalacetone guanylhydrazone hydrochloride
3	273	278.0	237	1000	W		P-dimethylamine benzaldehyde guanylhydrazone hydrochloride
4	276	233.0	170 ~ 171	1000	W		P-chlore benzaldehyde guanylhydrazone hydrochloride
5	277	243.5	244 ~ 246	1000	G		P-nitre benzaldehyde guanylhydrazone hydrochloride
6	278	212.5	202 ~ 203	1000	W		acetophenon guanylhydrazone hydrochloride
7	279	255.5	259 ~ 260	1000	G		p-acethylamine benzaldehyde guanylhydrazone hydrochloride
8	280	238.5	239	1000	G		Benzalacetone guanylhydrazone hydrochloride
9	281	162.0	178	1000	G		Benzaldehyde guanylhydrazone
10	282	360.5	198	500	G		α-brome-P-dimethylamno benzalacetone guanylhydrazone hydrochloride
11	283	257.5	280	1000	G		p-nitno-acetophenon guanylhydrazone hydrochloride

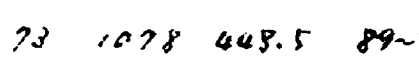
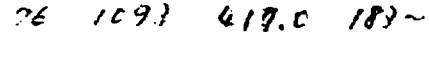
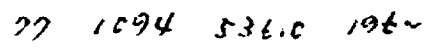
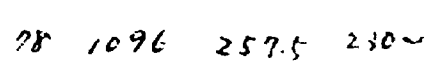
12	284	374.5	230	1000	G		α-bromo-P-acethylamine benzalacetone guanylhydrazone hydrochloride
13	285	295.5	233	1000	G		P-acethylamine benzalacetone guanylhydrazone hydrochloride
14	290	273.0	226	1000	W		P-chlore benzalacetone guanylhydrazone hydrochloride
15	292	269.5	271	1000	G		P-acethylamine acetophenone guanylhydrazone hydrochloride
16	293	352.0	213~214	500	G		P-chlore-α-bromebenzalacetone guanylhydrazone hydrochloride
17	296	245.0	210~211	500	G		P-dimethylamine benzalacetone guanylhydrazone hydrochloride
18	298	243.5	250~1	1000	G		m-nitre benzaldehyde guanylhydrazone hydrochloride
19	302	242.5	205~7	1000	G		P-methoxy acetophenon guanylhydrazone hydrochloride
20	304	267.0	246~7	1000	W		P-chlore acetophenon guanylhydrazone hydrochloride
21	327	283.5	213~4	1000	G		m-nitre benzalacetone guanylhydrazone hydrochloride
22	406	252.5	181~2	1000	G		α-methyl benzalacetone guanylhydrazone hydrochloride
23	418	317.5	214~5	1000	G		α-breme benzalacetone guanylhydrazone hydrochloride

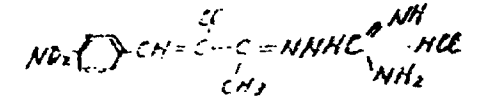
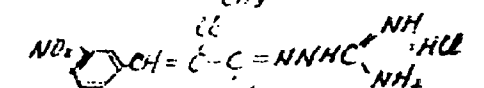
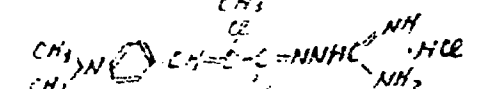
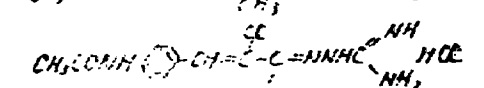
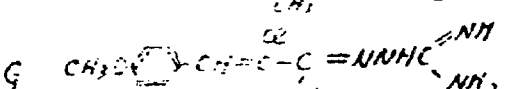
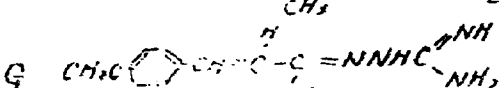
24	444	362.5	243~4	1000	G		α-bromo-p-nitro benzalacetone guanylhydrazone hydrochloride
25	622	297.5	244	500	G		α-methyl-p-nitic benzalacetone guanylhydrazone hydrochloride
26	901	272.0	190~1	1000	W		N',N'-anhydro bis(β-hydroxyethyl)-N²-aminoguanidine hydroiodide
27	902	184.0	60~64	1000	W		Acetone N',N'-anhydro bis(β-hydroxy-ethyl) guanylhydrazone
28	903	312.0	169~171	1000	W		Acetone N',N'-anhydrobis(β-hydroxy-ethyl) guanylhydrazone hydroiodide
29	904	359.0	53~55	1000	W		Acetone S-ethyl isothiosemi-carbazone
30	907	360	210.5~213	1000	W		Benzaldehyde N',N'-anhydrobis(β-hydroxyethyl) guanylhydrazone hydroiodide
31	909	233.0	114~6	1000	W		S-methyl isothiosemicarbazide hydroiodide
32	931	400.0	197~9	500	G		Benzalacetone N',N'-anhydro bis(β-hydroxy ethyl) guanylhydrazone hydroiodide
33	932	414.0	151	1000	W		α-methyl benzalacetone N',N'-anhydro bis (β-hydroxyethyl) guanylhydrazone hydroiodide
34	934	374.0	197~9	1000	W		Acetophenone N',N'-anhydro bis (β-hydroxyethyl) guanylhydrazone hydroiodide
35	960	415.0	164~5	1000	W		α-bromo cinnamaldehyde-N',N'-anhydro bis-(β-hydroxyethyl) guanylhydrazone hydroiodide

36	983	282.5	197.5~198	1000	W		α-methyl anisal acetone guanylhydrazone hydrochloride
37	987	207.0	116~7	1000	W		N',N'-anhydro bis (β-hydroxyethyl) N²-aminoguanidine nitrate
38	988	180.5	245~6	1000	W		N',N'-anhydro bis (β-hydroxyethyl) N²-aminoguanidine hydrochloride
39	990	293.0	141.5~2	1000	W		Benzaldehyde N',N'-anhydro bis (β-hydroxyethyl) guanyl hydrazone
40	991	268.5	209.5~212.5	1000	W		Benzaldehyde N',N'-anhydro bis (β-hydroxyethyl) guanylhydrazone hydrochloride
41	992	231.0	112~112.5	1000	W		Acetophenone N',N'-anhydro bis (β-hydroxyethyl) guanylhydrazone
42	993	282.5	211~212	1000	W		Acetophenone N',N'-anhydro bis (β-hydroxyethyl) guanylhydrazone hydrochloride
43	994	220.5	79~81	1000	W		Acetone N',N'-anhydro bis (β-hydroxyethyl) guanylhydrazone hydrochloride
44	997	273.0	172.3~173	1000	W		Acetone N-methyl isothiosemicarbazone hydriodide.
45	1030	297.5	242~3	1000	G		α-methyl-m-nitro benzalacetone guanylhydrazone hydrochloride
46	1046	309.5	232	1000	G		α-methyl-p-acetaminobenzalacetone guanylhydrazone hydriodide.
47	1050	312.0	209~210	1000	W		α-methyl-p-dimethylamino benzalacetone guanylhydrazone hydrochloride

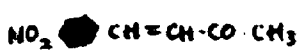
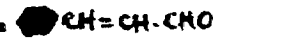
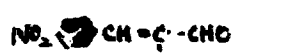
48	1051	326.0	188-190	1000	W		Methyl ethyl ketone N,N'-anhydro bis[(β-hydroxyethyl) guanylhydrazone hydroiodide
49	1052	417.0	259-260	1000	W		P-acetoamino-benzaldehyde N,N'-anhydro bis[(β-hydroxyethyl) guanylhydrazone hydroiodide
50	1053	405.0	221	1000	W		P-nitro benzaldehyde N,N'-anhydro bis[(β-hydroxyethyl) guanylhydrazone hydroiodide
51	1054	459.0	197.2	1000	W		P-nitro-α-methyl benzalacetone N,N'-anhydro bis[(β-hydroxyethyl) guanylhydrazone hydroiodide
52	1055	403.0	206-208	1000	W		P-dimethylamino benzaldehyde N,N'-anhydro bis[(β-hydroxyethyl) guanylhydrazone hydroiodide
53	1056	376.0	189-192	1000	W		Salicylaldehyde N,N'-anhydro bis[(β-hydroxyethyl) guanylhydrazone hydroiodide
54	1057	386.0	210-211	1000	W		Cinnamaldehyde N,N'-anhydro bis[(β-hydroxyethyl)] guanylhydrazone hydroiodide
55	1058	479.0	145-7	1000	W		α-bromo benzalacetone N,N'-anhydro bis[(β-hydroxyethyl)] guanylhydrazone hydroiodide
56	1059	445.0	242	500	G		P-nitro benzalacetone N,N'-anhydro bis[(β-hydroxyethyl)] guanylhydrazone hydroiodide
57	1062	431.0	202-3	1000	G		P-nitro cinnamaldehyde N,N'-anhydro bis[(β-hydroxyethyl)] guanylhydrazone hydroiodide
58	1063	408.5	214-5	1000	W		P-chloro acetophenone N,N'-anhydro bis[(β-hydroxyethyl)] guanylhydrazone hydroiodide
59	1064	434.5	222	1000	W		P-chloro benzalacetone N,N'-anhydro bis[(β-hydroxyethyl)] guanylhydrazone hydroiodide

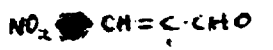
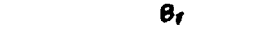
60	1065	513.5	169	500	G		p-chloro α-methyl benzalacetone N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide
61	1066	443.0	194~5	1000	W		p-dimethylamino benzalacetone N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide
62	1067	457.0	233	1000	W		p-acetamino benzalacetone N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide
63	1068	457.0	176	1000	G		p-dimethylamino α-methyl benzalacetone N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide
64	1069	471.0	219	1000	W		p-acetamino α-methyl benzalacetone N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide
65	1070	419.0	194~5	1000	W		p-nitro acetophenon N,N'-anhydrobis-(β-hydroxyethyl) guanylhydrazone hydriodide
66	1071	394.5	230~1	1000	W		p-chloro benzaldehyde N,N'-anhydro bis-(β-hydroxyethyl) guanylhydrazone hydriodide
67	1072	510.0	148~9	1000	G		p-nitro-α-bromo cinnamaldehyde N,N'-anhydro-bis (β-hydroxyethyl) guanylhydrazone hydriodide
68	1073	524.0	50~53.9	1000	G		p-nitro-α-bromobenzalacetone N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide
69	1074	429	196	1000	G		p-dimethylamino cinnamaldehyde N,N'-anhydro-bis (β-hydroxyethyl) guanylhydrazone hydriodide
70	1075	508.0	178	1000	G		p-dimethylamino-α-bromocinnamaldehyde N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide
71	1076	431.0	246~7	1000	W		p-acetamino acetophenon N,N'-anhydrobis (β-hydroxyethyl) guanylhydrazone hydriodide

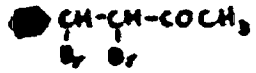
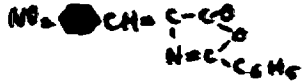
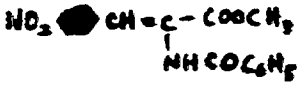
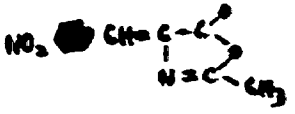
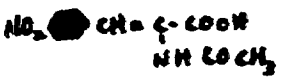
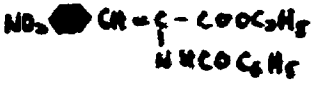
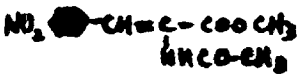
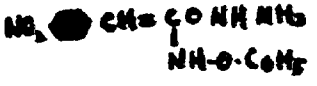
72	1077	443.0	272.5	1000	W		p-acetamino cinnamaldehyde-N,N'-anhydro-bis(β hydroxyethyl) guanylhydrazone hydrochloride
73	1078	448.5	89~91	1000	G		p-chloro-α-methylbenzalacetone N,N'anhydro-bis(β-hydroxyethyl) guanylhydrazone hydroiodide
74	1079	287.0	213.5	1000	G		α-methyl-p-chlorobenzalacetone guanylhydrazone hydrochloride
75	1081	522.0	156	500	G		p-dimethylamino-α-bromobenzalacetone N,N anhydrobis(β hydroxyethyl) guanylhydrazone hydroiodide
76	1093	419.0	183~5	1000	W		p-dimethylamino acetophenone N,N'anhydro bis(β-hydroxyethyl) guanylhydrazonehydroiodide
77	1094	536.0	196~7	[illegible]	G		α-bromo-p methylamino benzalacetone N,N'anhydrobis(β-hydroxyethyl) guanylhydrazone hydroiodide
78	1096	257.5	230~1	1000	G		m-nitro acetophenone guanylhydrazone hydrochloride
79	1097	292.0	223~230	1000	W		p-dimethylamino acetophenone guanylhydrazone hydrochloride
80	1098	192.0	185	1000	G		p-methoxy benzaldehyde guanylhydrazone
81	1099	347.5	196	1000	G		α-bromo-p-methoxy benzalacetone guanylhydrazone hydrochloride
82	1100	273.0	217.5~218	1000	W		α-chloro benzalacetone guanylhydrazone hydrochloride
83	1101	307.5	231~2	1000	W		α-chloro-p-chloro benzalacetone guanylhydrazone hydrochloride

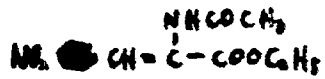
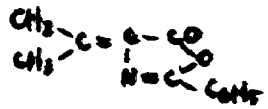
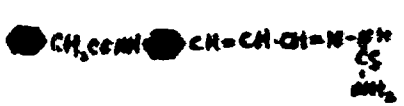
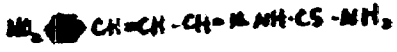
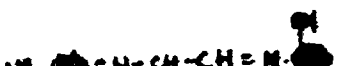
84	1102	318.0	251~2	1000	G		α-chloro-p-nitro benzalacetone guanylhydrazone hydrochloride
85	1103	318.0	253	1000	G		α-chloro-m-nitro benzalacetone guanylhydrazone hydrochloride
86	1104	316.0	175~6	500	G		α-chloro-p-dimethylamino benzal-acetone guanylhydrazone hydrochloride
87	1105	330.0	236	1000	G		α-chloro-p-acethylamino benzal-acetone guanylhydrazone hydrochloride
88	1106	266.5	183	500	G		α-chloro-p-methoxy benzalacetone guanylhydrazone hydrochloride
89	1107	232.0	211.5~212	500	G		p-methoxy benzalacetone guanylhydrazone hydrochloride

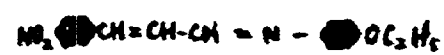
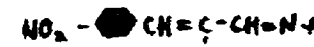
Note : Solvent, G = 50% glycerin, W = distilled water.

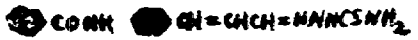
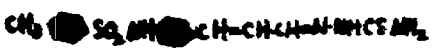
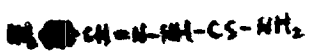
	Group	B– carbonyl					
90	1	191	109–110	1000	G		p-Nitro benzalacetone
91	2	177	141–142	1000	G		p-Nitrocinnamaldehyde
92	5	191	155–158	1000	G		o-(β.β-dimethylacrylylamino) phenol
93	6	226	55–6	1000	G		p-Nitro cinnamyl acetate
94	9	256	137.5–138	1000	G		p-Nitro- -bromocinnamaldehyde
95	23	163	156–7	1000	G		p-Acetylaminobenzaldehyde
96	29	253	128	1000	G		p-Nitrobenzal diacetate

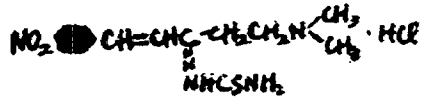
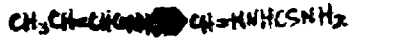
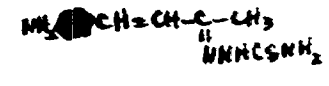
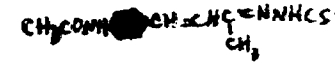
97	30	151	106-107.5	1000	G		p-Nitrobenzaldehyde
98	34	139	177	1000	G		p-Acetylaminocinnamaldehyde
99	69	256	136.5-137	1000	G		p-Nitro-α-bromo cinnamaldehyde
100	71	270	120	1000	G		p-Nitro-α-bromobenzalacetone
101	73	551	128	1000	G		p-Nitrobenzalacetone α,-β dibromide
102	120	1777	169-170	1000	G		p-Acetylaminoacetophenone
103	121	198	52-53	1000	G		Furfurylidene diacetate
104	125	165	82-82.5	1000	G		p-Nitroacetophenone
105	137	253	112-3	1000	G		Distyrylketone

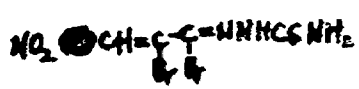
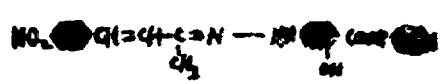
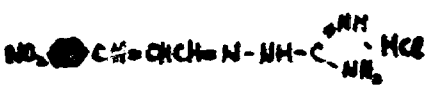
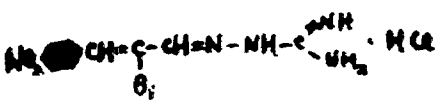
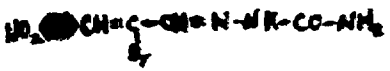
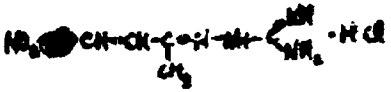
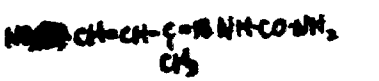
106	148	135	105.5 -6.5	1000	G		p-Amino acetophenone
107	198	306	125	1000	G		d-β-Dibromo benzalacetone
	Group C-azlactone						
108	7	294	238	1000	G		2-phenyl-4-(p-nitrobenzal) azlactone
109	8	326	192	1000	G		Methyl α-(p-nitro) benzalhippurate
110	10	232	184-5	1000	G		4-p-Nitrobenzal-2-methyl-5-exazolone
111	17	250	210	1000	G		d-Acetylamino-p-nitro cinnamic acid
112	165	321	167-8	1000	G		Ethyl-d-benzalamino p-nitro-cinnamate
113	166	264	175-6	1000	G		Methyl-d-acetylamino p-nitro cinnamate
114	167	303	243-4	1000	G		d-Benzoylamino-p-nitrcinnamic acid hydrazide

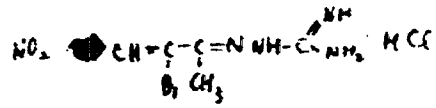
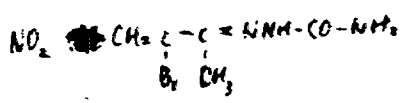
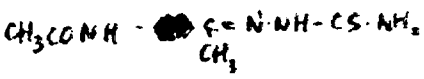
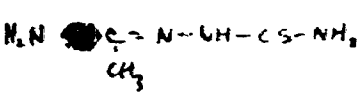
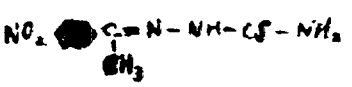
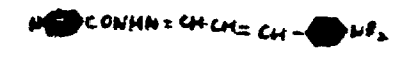
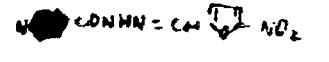
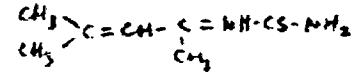
115	194	278	178	1000	G		Ethyl-α-acethylamino-p-nitro cinnamate
116	196	201	99	1000	G		4-Isopropylidene-2-phenyl-5-omazolone
	Group D-hydrazone						
117	12	234	223	1000	G		p-Nitrocinnamaldehyde semicarbasone
118	15	338	202	1000	G		p-Phenylacetamido-cinnamaldehyde thio-semicabazone
119	18	236	207	1000	G		Tibion
120	19	250	207	1000	G		p-Nitro cinnamaldehyde thiosemicarbazone
121	20	268	152-153	1000	G		p-Nitrocinnamaldehyde-o-hydroxyanil
122	22	76	169-170	1000	G		Thiourea
123	24	220	205	1000	G		p-Aminocinnamaldehyde thiosemicarbazone

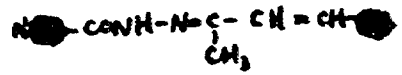
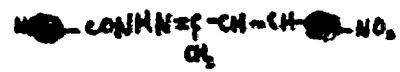
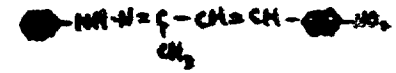
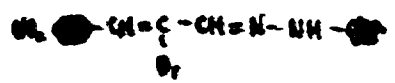
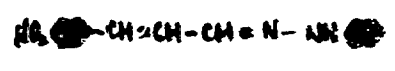
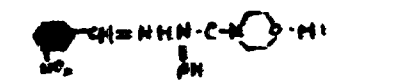
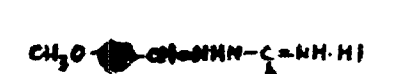
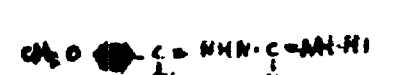
124	26	262	222-5	1000	G		p-Acetylamino-cinnamaldehyde thiosemicarbazone
125	27	296	139-142	1000	G		p-Nitrocinnamaldehyde p-ethoxyanil
126	31	329	207	1000	G		4-Bromo-p-nitrocinnamaldehyde thiosemicarbazone
127	32	261	225	1000	G		p-Acetylaminocinnamaldehyde thiosemicarbazone
128	33	117	147-147.5	1000	G		Acetaldehyde thiosemicarbazone
129							
130							
131	37	264	240	1000	G		p-Nitrobenzalacetone thiosemicarbazone
132	39	290	223	1000	G		p-1-methylbutylaminocinnamaldehyde thiosemicarbazone

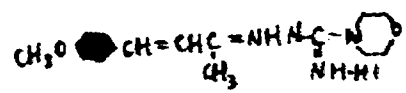
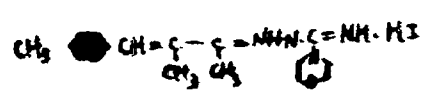
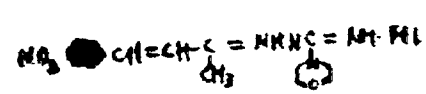
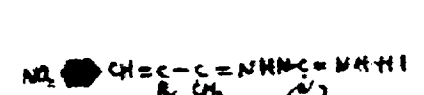
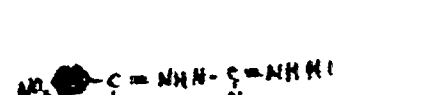
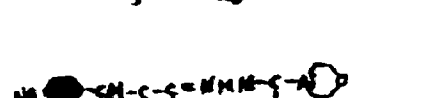
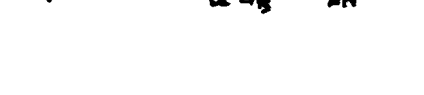
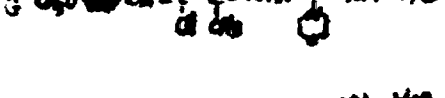
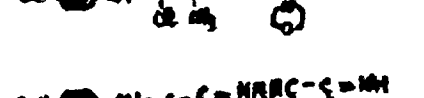
133	43	351	229	1000	G		p-Cinnamoylaminocinnamaldehyde thiosemicarbazone
134	44	324	218	1000	G		p-Benzoylaminocinnamaldehyde thiosemicarbazone
135	45	374	216-218	1000	G		p-Tosylaminocinnamaldehyde thiosemicarbazone
136	46	302		1000	G		p-(3-Methyl-2-butanoyl) amino cinnamaldehyde thiosemicarbazone
137	48	224	234	1000	G		p-Nitrobenzaldehyde thiosemicarbazone
138	49	271	220	1000	G		p-(3-methy-2-butenoyl) amino benzaldehyde thiosemicarbazone
139	51	318	212-213	1000	G		p-Phenylacetylaminocinnamaldehyde thiosemicarbazone
140	54	293	201	1000	G		p-Hexanoylaminobenzaldehyde thiosemicarbazone
141	55	318	198-9	1000	G		p-Hexanoylaminocinnamaldehyde thiosemicarbazone

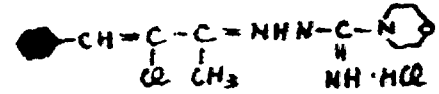
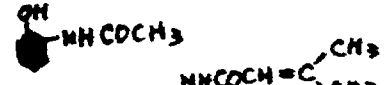
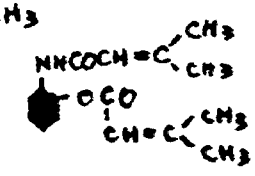
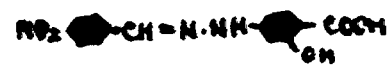
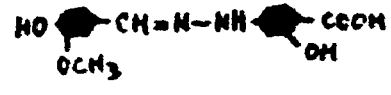
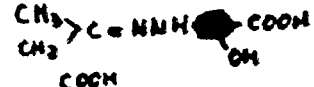
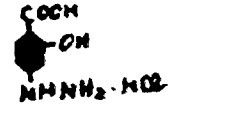
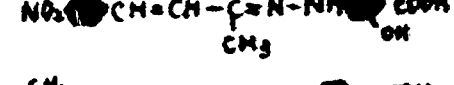
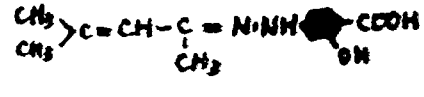
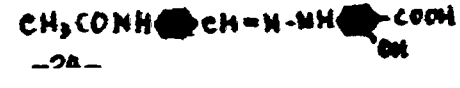
142	57	194	197-198	1000	G		p-Aminobenzaldehyde thiosemicarbazone
143	59	357.5	205	1000	G		1-p-Nitrophenyl-5-dimethylamino pentene-1-one-3-thiosemicarbazone hydrochloride
144	61	236	230	1000	G		p-Acetylamino benzaldehyde thiosemicarbazone
145	62	294	216	1000	G		pp Succinoylaminobenzaldehyde thiosemicarbazone
146	63	262	236	1000	G		p-Crotonylaminobenzaldehyde thiosemicarbazone
147	64	288	235	1000	G		p-Crotonylaminicinnamaldehyde thiosemicarbazone
148	65	262	214	1000	G		p-Methacrylaminobenzaldehyde thiosemicarbazone
149	66	234	190	1000	G		p-Aminobenzalacetone thiosemicarbazone
150	67	276	220-227	1000	G		p-Acetylaminobenzalacetone thiosemicarbazone

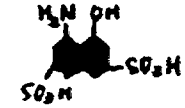
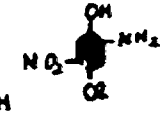
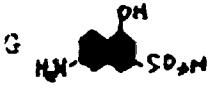
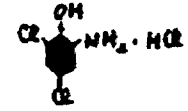
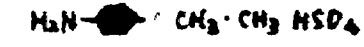
151	74	408	191 d.	1000	G		p-Nitro-α-bromobenzalacetone Thiosemicarbazone
							Nitroguanidine
152	77	103	244 d.	1000	G		Nitroguanidine
153	78	136	172-173	1000	G		Aminoguanidine bicarbonate
154	97	421	221-222	1000	G		Pyridiniump-nitro benzal acetone 3-hydroxy phenylhydrazone-4-carboxylate
155	107	269.5	238	1000	G		p-Nitrocinnamaldehyde guanylhydrazone hydrochloride
156	111	348	265 d.	1000	G		p-Nitro-α-bromocinnamaldehyde guanylhydrazone hydrochloride
157	113	313	223 d.	1000	G		p-Nitro-α-bromocinnamaldehyde semicarbasone
158	1115	283	270 d.	1000	G		p-Nitrobenzalacetone guanylhydrazone hydrochloride
159	116	248	235 d.	1000	G		p-Nitrobenzalacetone semicarbazone

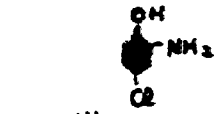
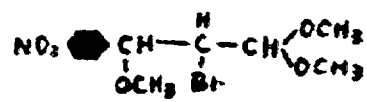
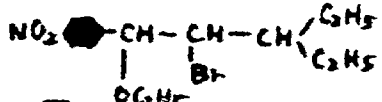
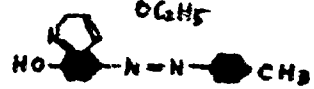
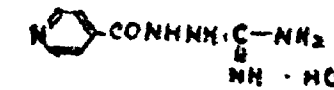
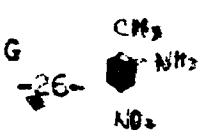
160	117	362.5	249-50	1000	G		p-Nitro-d-bromobenzalacetone guanyl hydrazone hydrochloride
161	118	327	229	1000	G		p-Nitro-d-bromobenzalacetone semicarbazone
162	123	250	225	1000	G		p-Acetylaminoacetophenone thiosemicarbazone
163	124	208	185	1000	G		p-Aminoacetophenone thiosemicarbazone
164	126	238	240 d.	1000	G		p-Nitroacetophenone thiosemicarbazone
165	127	218	215-216	1000	G		Furfural isonicotinoyl hydrazone
166	128	296	246 d.	1000	G		p-Nitrocinnamaldehyde isonicotinoyl hydrazone
167	129	260	250 d.	1000	G		5-Nitrofurfural isonicotinoyl hydrazone
168	133	157	148-149	1000	G		4-Methylpentene 3-one-2 thiosemicarbazone

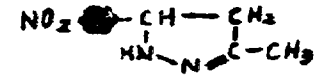
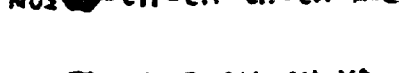
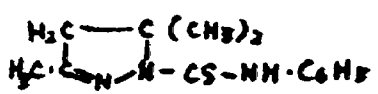
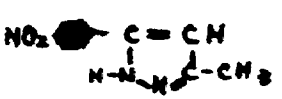
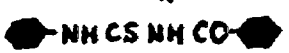
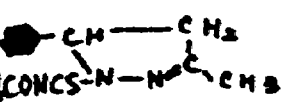
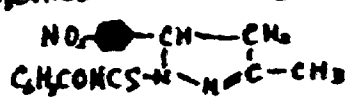
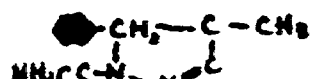
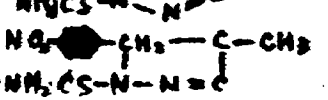
169	149	265	186-187	1000	G		Benzalacetone isonicotinoyl hydrazone
170	150	310	256-257	1000	G		p-Nitrobenzalacetone isonicatinoyl hydrazone
171	152	281	193-196	1000	G		p-Nitro benzalacetone phenyl hydrazone
172	158	379	155 d.	1000	G		p-Nitro-d-bromocinnamaldehyde phenyl hydrazone
173	159	267	181-181.5	1000	G		p-Nitrocinnamaldehyde phenylhydrazone
174	195	115	187-188	1000	G		Acetone semicarbazone
175	1112	405	216-219	1000	G		m-nitrobenzaldehyde N;N'-anhydrobis(β-hydroxyethyl)-guanylhydrazone hydroiodide
176	1115	390	116-117	1000	G		p-methoxybenzaldehyde N;N'-anhydrobis (β-hydroxyethyl)-guanylhydrazone hydroiodide
177	1119	405	172-173	1000	G		p-methoxyacetophenone N;N'-anhydrobis(β-hydroxyethyl)-guanylhydrazone hydroiodide

178	1120	430	206-207.5	1000	G		p-methoxybenzalacetone N¦N'-anhydrobis (β-hydroxyethyl)guanylhydrazone hydroiodide
179	1121	428	152-153	1000	G		- methylanisalacetone-N¦N'-anhydrobis(β-hydroxyethyl)-guanylhydrozone-hydroiodide
180	1123	445	230	1000	G		m-nitrobenzalacetone N¦N'-anhydrobis-(β-hydroxyethyl)-guanyl hydrazone hydroiodide
181	1124	524	181.5	1000	G		m-nitro-α'-bromobenzalacetone N¦N'-anhydrobi. (β-hylroxyethyl)-guanylhydrazonehydroiodide
182	1156	419	203-205	1000	G		m-nitroacetophenone N¦N'-anhydrobis(β-hydroxyethyl)-guanyl hydrazone hydrochloride
183	1157	3888	202-203	1000	G		α- chloro-m-nitrobenzalacetone N¦N'-anhydrobis(β-hydroxyethyl)guanyl hydrazone hydrochloride
184	1165	400	252	1000	G		α- chloro-p-acetylaminobenzalacetone N¦N'-anhydrobis(β-hydroxyethyl) guanylhydrazone hydrochloride
185	1167	373	188-190	1000	G		p-methoxy- -chlorobenzalacetone N¦N'-anhydrobis(β-hydroxyethyl)guanyl hydrazone hydrochloride
186	1168	378	193-194	1000	G		p-chloro- -chlorobenzalacetone N¦N'-anhydrobis(β-hydroxyethyl) guanyl hydrazone hydrochloride
187	1169	364	167	1000	G		α-chloro-p-nitrobenzalacetone N¦N'-anhydrobis(β-hydroxyethyl) guanylhydrazone

189	1172	343	208-209	1000	G		d-chloro benzalacetone-N,N-anhydro-bis(B-hydroxyethyl)-guanylhydrosone hydrochloride
	Group E-phenol						
190	52	109	173	1000	G		o-Aminophenol
191	53	151	192	1000	G		o-hydroxy acetanilide
192	56	273	104.5-5	1000	G		N,o-bis(3-methyl-2-butenoyl)-o-aminophenol
193	60	145	75	1000	G		8-Hydroxyquinoline
194	76	154	213	1000	G		3.4-dihydroxybenzoic acid
195	91	270	19?-199	1000	G		Sodium 3-Hydroxy-4-carboxyphenyl hydrozo sulfonate
196	92	168	148-149	1000	G		P-Hydroxy-4-hydrazinobenzoic acid
197	93	301	228-229	1000	G		p-Nitrobenzaldehyde 3-hydroxy-4-carboxyphenyl hydrazone
198	94	302	195	1000	G		Vaniline 3-hydroxy-4-carboxy phenyl hydrozone
199	95	208	198-199	1000	G		Acetone 3-hydroxy-4-carboxy phenylhydrazone
200	96	204.5	177	1000	G		3-hydroxy-4-carboxyphenyl hydrazine hydrochloride
201	99	341	221-222	1000	G		p-Nitrobenzalacetone 3-hydroxy -4-carbody phenyl hydrazone
202	103	248	173	1000	G		4-Methylpentene-3-one-2-m-hydroxy -p-carboxy phenyl hudrazone
203	110	313	213	1000	G		p-Acetylaminobenzaldehyde-3-hydroxy -4-carboxy phenyl hydrozone

204	168	137	46	1000	J		p-methyl-m-amino-anisol -
205	169	154	77	1000	G		p-Nitro-opaminophenol
206	170	168	138-140	1000	G		5-Nitro-2-aminoanisol
207	171	155	201-202	1000	G		5-Nitro-2-aminophenol
208	172	319	280	1000	G		3-Amino-1-naphtol-3,6 disulfonic acid
209	173	188.5	234	1000	G		5-Nitro-4-chloro-2-amino phenol
210	175	239	290	1000	G		6-Amino-1-naphthol-3-sulfonic acid
211	176	154		1000	G		4-Nitro-2-amino phenol
212	178	214.5	235-240	1000	G		4,6-dichloro-2-aminophenol hydrochloride
213	180	168		1000	G		p-Nitro-o-anisidine
214	182	239	293	1000	G		2-Amino-8-naphthol-6-sulfonic acid
215	184	244	290(d)	1000	G		Dianisidine-HCl salt
216	185	199	168-168:2	1000	G		Dicromic acid
217	186	154	200	1000	G		5-Nitro-2-aminophenol
218	187	235	260	1000	G		Mono methyl-p-aminophenol sulfate
219	189	108	285	1000	G		3-Amino-4-hydroxybenesulfonic acid

220	192	143.5	136.2-.5	1000	G		4-Chloro-2-aminophenol
221	193	159	160	1000	G		1-Amino-2-naphthol
Group F-miscellaneous							
222	3	179	125-127	1000	G		p-Nitrocinnamyl alcohol
223	4	334	59-63	1000	G		α-bromo-β-(p-nitrophenyl-β-methoxy-propionaldehyde dimethyl acetal
224	33	344	149-150	1000	G		α-Bromo-β-ethoxy-p-Nitro hydro-cinnamaldehyde diethylacetal
225	50	263	190	1000	G		5-(p-Tolylazo)-8-hydroxy quinoline
226	58	284.5	186-187	1000	G		1-p-Nitrophenyl-3-dimethyl amino pentene-1-one-3 hydrochloride
227	72	215.5	300	1000	G		Isonicotinoylaminoguanidine hydrochloride
228	79	205	250-251	1000	G		p-Acetylaminocinnamic acid
229	81	178	218	1000	G		p-Hydrazinocinnamic acid
230	84	167	238-239	1000	G		p-Nitrobenzoic acid
231	85	137	185-186.5	1000	G		p-Aminobenzoic acid
232	86	163	167	1000	G		p-Aminocinnamic acid
233	87	193	285	1000	G		p-Nitrocinnamic acid
234	88	152	108.5-109	1000	G		2-Amino-4-nitro toluene

235	102	205	103-105 100-101 d.	1000	G		3-Methyl-5-p-nitrophenyl pyrazoline
236	106	191	145-147	1000	G		p-Nitrocinnamaldehyde hydrazone
237	108	194	203-204 d.	1000	G		4,β-dinitrostyrene
238	109	220	200-201 d.	1000	G		1-p-Nitrophenyl-4-nitrobutadiene-1,3
239	114	299	182-182.5	1000	G		1-p-Nitrophenyl-2-bromo-4-nitro-1,3-butadiene
240	130	171	235-235	1000	G		p-Nitrobenzoic acid
241	131	195	54-56	1000	G		Ethyl p-nitrobenzoate
242	134	247	103-104	1000	G		1-Phenylthiocarbamoyl-3-methyl-5,5-dimethylpyrazoline
243	135	203	191-192	1000	G		3-Methyl-5-p-nitrophenyl pyrazole
244	141	256	146-147	1000	G		α-Benzoyl-β-phenyl thiourea
245	142	152	155-156	1000	G		Phenylthiaurea
246	143	322	185-5.5 d.	1000	G		1-Benzoylthiocarbamoyl-3-methyl-5-phenyl pyrazoline
247	144	367	199-200	1000	G		1-Benzoylthiocarbamoyl-3-methyl-5-p-nitropheryl pyrazoline
248	145	194	170-171	1000	G		α-Acetyl-βphenylthiourea
249	146	218	230-231	1000	G		1-Thiocarbamoyl-3-methyl-5-phenylpyrazoline
250	147	263	271	1000	G		1-Thiocarbamoyl-3-methyl-5-p-nitrophentl pyrazoline

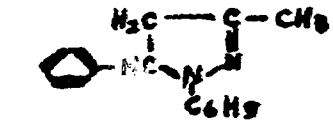
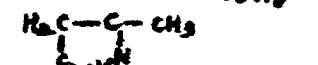
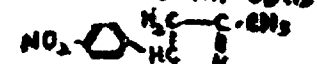
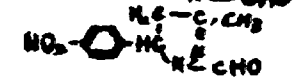
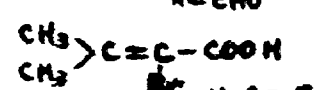
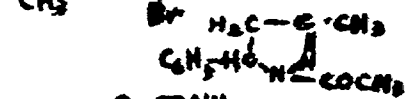
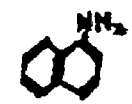
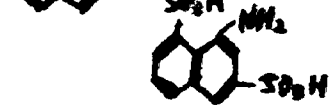
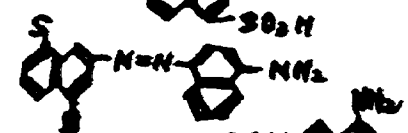
251	153	236	112-113	1000	G		3-methyl-1,5-dipheryl pyrazoline
252	154	280	113-3.5	1000	G		1-Phenyl-3-methyl-5-p-nitro-phenyl pyrazoline
253	160	217	160-161	1000	G		1-Phenylthiocarbamoyl-3-methyl -5-phenyl pyrazoline
254	161	233	121-122	1000	G		1,3-methyl-5-nitro-formyl phenylpyrazoline
255	162	188	88.5-90	1000	G		1-Formyl-3-methyl-5-phenyl pyrazoline
256	163	179	90-91	1000	G		d- Bromo-β-methyl-crotonic acid
257	164	202	77.5-78	1000	G		1-Acethyl-3-methyl-5-phenylpyrazoline
258	174	223	290	1000	G		1-naphthylamino-6-sulfonic acid
259	177	143	169.5-170.5	1000	G		d-naphtyl amine
260	179	303	293	1000	G		1-Aminonaphtyl-3,8-di-sulfonic-acid-mono Na salt.
261	181	303	293	1000	G		2-Aminonaphthaline-5,7 di-sulfonic acid
262	183	303	300	1000	G		1-Aminonaphthaline-4,8-disulfonic acid mono sodium salt
263	188	360	3000	1000	G		2-(4-aminonaphtyl)azo-naphthalene -4,8-disulfonic acid
264	190	223	285	1000	G		1-Naphthylamine-7-sulfonic acid
265	191		123	1000	G		p-amino diphenylamine diazonium salt

Table 11. Screening test of chemical compounds in chick chorioallantoic membrane culture

Serial No.	Synthesis No.	Concentration[§]	HA[§§] percent	Serial No.	Synthesis No.	Concentration[§]	HA[§§] percent
1	115	100	0	39	990	100	50
2	117	100	0	40	991	100	50
3	273	100	0	41	992	100	25
4	276	100	0	42	993	100	50
5	277	100	0	43	994	100	100
6	278	100	0	44	997	100	50
7	279	100	50	45	1030	100	0
8	280	100	100	46	1046	100	0
9	281	100	0	47	1050	100	0
10	282	50	12.5	48	1051	100	50
11	283	100	0	49	1052	100	100
12	284	100	0	50	1053	100	50
13	285	100	25	51	1054	100	0
14	290	100	0	52	1055	100	6.3
15	292	100	50	53	1056	100	100
16	293	50	0	54	1057	100	12.5
17	296	50	12.5	55	1058	100	0
18	298	100	0	56	1059	50	0
19	302	100	0	57	1062	100	0
20	304	100	0	58	1063	100	6.3
21	327	100	0	59	1064	100	0
22	406	100	0	60	1065	50	100
23	418	100	0	61	1066	100	50
24	444	100	0	62	1067	100	100
25	622	50	0	63	1068	100	100
26	901	100	50	64	1069	100	50
27	902	100	50	65	1070	100	50
28	903	100	12.5	66	1071	100	25
29	904	100	50	67	1072	100	0
30	907	100	100	68	1073	100	12.5
31	909	100	50	69	1074	100	0
32	931	50	50	70	1075	100	50
33	932	100	12.5	71	1076	100	50
34	934	100	50	72	1077	100	100
35	960	100	0	73	1078	100	0
36	983	100	0	74	1079	100	0
37	987	100	50	75	1081	50	12.5
38	988	100	100	76	1083	100	50

Serial No.	Synthesis No.	Concentration	HA percent
77	1094	100	0
78	1096	100	0
79	1097	100	12.5
80	1098	100	0
81	1099	100	0
82	1100	100	0
83	1101	100	0
84	1102	100	0
85	1103	100	0
86	1104	50	0
87	1105	100	0
88	1106	50	6.3
89	1107	50	0
	Group B		
90	1	100	0
91	2	100	0
92	5	100	100
93	6	100	100
94	9	100	0
95	28	100	100
96	29	100	100
97	30	100	25
98	34	100	0
99	69	100	6.3
100	71	100	0
101	73	100	0
102	120	100	100
103	121	100	100
104	125	100	100
105	137	100	25
106	148	100	100
107	198	100	0
	Group C		
108	7	100	100
109	8	100	100
110	10	100	100
111	17	100	100
112	165	100	100
113	166	100	100
114	167	100	100
115	194	100	100
116	196	100	100
	Group D		
117	12	100	100
118	15	100	50
119	18	100	100
120	19	100	100
121	20	100	100
122	22	100	0
123	24	100	100
124	26	100	50
125	27	100	50
126	31	100	12.5
127	32	100	100
128	33	100	25
129	35	100	0
130	36	100	0
131	37	100	100
132	39	100	12.5
133	43	100	100
134	44	100	100
135	45	100	50
136	46	100	50
137	48	100	100
138	49	100	100
139	51	100	100
140	54	100	100
141	55	100	100
142	57	100	25
143	59	100	0
144	61	100	100
145	62	100	50
146	63	100	100
147	64	100	100
148	65	100	50
149	66	100	0
150	67	100	100
151	74	100	0
152	77	100	100
153	78	100	100

Serial No.	Synthesis No.	Concentration	HA percent
154	97	100	0
155	107	100	0
156	111	100	0
157	113	50	0
158	115	100	0
159	116	100	100
160	117	100	0
161	118	100	100
162	123	100	100
163	124	100	100
164	126	100	100
165	127	100	100
166	128	100	100
167	129	50	0
168	133	100	100
169	149	100	0
170	150	100	100
171	152	100	100
172	158	50	0
173	159	100	100
174	195	100	100
175	1112	100	50
176	1115	100	100
177	1119	100	100
178	1120	100	0
179	1121	100	12.5
180	1123	100	50
181	1124	100	25
182	1156	100	100
183	1157	100	0
184	1165	100	0
185	1167	100	25
186	1168	50	0
187	1169	50	0
189	1172	100	0
	Group D		
190	52	100	0
191	53	100	50
192	56	100	25
193	60	100	0
194	76	100	100
195	91	100	100
196	92	100	0
197	93	100	100
198	94	100	0
199	95	100	0
200	96	100	100
201	99	50	0
202	103	100	100
203	110	50	0
204	167	100	100
205	169	100	25
206	170	100	50
207	171	100	25
208	172	100	100
209	173	50	0
210	175	100	0
211	176	100	50
212	178	100	0
213	180	100	100
214	182	100	0
215	184	100	0
216	185	100	0
217	186	100	12.5
218	187	100	0
219	189	100	0
220	192	100	0
221	193	100	0
	Group F		
222	3	100	100
223	4	100	50
224	38	100	0
225	50	100	50
226	58	50	0
227	72	100	100
228	81	100	100

Serial No.	Synthesis No.	Concentration	HA percent	Serial No.	Synthesis No.	Concentration	HA percent
229	79	100	100	248	145	100	100
230	84	100	100	249	146	100	100
231	85	100	100	250	147	100	100
232	86	100	100	251	153	50	0
233	87	100	100	252	154	50	12.5
234	88	100	50	253	160	100	100
235	102	100	100	254	161	100	100
236	106	100	100	255	162	100	50
237	108	50	0	256	163	100	0
238	109	50	0	257	164	100	100
239	114	50	0	258	174	100	0
240	130	100	50	259	177	100	0
241	131	100	100	260	179	100	0
242	134	100	100	261	181	100	0
243	135	100	100	262	183	100	0
244	141	100	100	263	188	100	100
245	142	100	100	264	190	100	0
246	143	100	100	265	191	100	0
247	144	100	100				

$: Final concentration of compound solution, γ/cc

3$: HA percent of control.

TABLE III Result of Screening test of 265 compounds

Inhibitory	Partially inhibitory	Non-inhibitory
Group A		
1, 2, 3, 4, 5, 6, 9, 11, 12, 14, 15, 18, 19, 20, 21, 22, 23, 24, 25, 35, 36, 45, 46, 47, 51, 55, 56, 57, 59, 67, 69, 73, 74, 77, 78, 80, 81, 82, 83, 84, 85, 86, 87, 88. Total 44	7, 10, 13, 15, 17, 26, 27, 28, 29, 31, 32, 33, 34, 37, 39, 40, 41, 42, 44, 48, 50, 52, 54, 58, 61, 64, 65, 66, 68, 70, 71, 75, 76, 79, 88. Total 35	8, 30, 38, 43, 49, 53, 60, 62, 64, 72. Total 10
Group B		
90, 91, 94, 98, 100, 101, 107. Total 7	97, 99, 105. Total 3	92, 93, 95, 96, 102, 103, 104, 106. Total 8
Group C		
-	-	108, 109, 110, 111, 112, 113, 114, 115, 116. Total 9
Group D		
122, 129, 130, 143, 149, 151, 154, 155, 156, 157, 158, 160, 167, 169, 172, 178, 183, 184, 186, 187, 189. Total 22	118, 124, 125, 126, 128, 132, 135, 136, 142, 145, 148, 175, 179, 180, 181, 185. Total 16	117, 119, 120, 121, 123, 127, 131, 133, 134, 137, 138, 139, 140, 141, 144, 146, 147, 150, 152, 153, 159, 161, 162, 163, 164, 165, 166, 168, 170, 171, 173, 174, 176, 177, 182. Total 35
Group E		
190, 193, 196, 198, 199, 201, 203, 209, 210, 212, 214, 215, 216, 218, 219, 220. Total 17	191, 192, 205, 206, 207, 211, 217. Total 7	194, 195, 197, 200, 202, 204, 208, 213. Total 8

Group F		
224, 226, 237, 238, 239, 251, 256, 258, 259, 260, 261, 262, 264, 265.	223, 225, 234, 240, 252, 255.	222, 227, 228, 229, 230, 231, 232, 233, 235, 236, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 253, 254, 257, 263.
Total 14	Total 6	Total 24

TABLE IV Summary of screening test.

Group of compound	Inhibitory		Partially inhibitory		Non-inhibitory		Total
	No.*	%	No.*	%	No.*	%	
A	44	49.4	35	39.3	10	11.2	89
B	7	38.8	3	16.6	8	44.6	18
C	0	0	0	0	9	100	9
D	22	30.1	16	21.9	35	47.9	73
E	17	53.1	7	21.8	8	25	32
F	14	31.8	6	13.6	24	54.5	44
Total	104	39.2	67	25.2	94	35.4	265

No.* : Numbers of chemical compounds.

TABLE V Quantitative analysis of antiviral and cytotoxicity tests in membrane culture

Serial No.	Synthesis No.	Antiviral activity[1] 100	50	25	12.5	Cytotoxic activity[1] 100	50	25	12.5	Ratio[2]
Group A										
1	115	0	0	6.3	50	0	6.3	50	100	1
2	117	0	0	0	12.5	0	0	100	100	1
3	273	0	0	0	100	0	100	100	100	2
4	276	0	0	6.3	25	0	25	100	100	1
5	277	0	0	25	50	50	100	100	100	≥2
11	283	0	0	0	12.5	50	100	100	100	≥4
12	284	0	0	6.3	50	0	25	100	100	1
14	290	0	0	0	6.3	0	0	12.5	100	1
16	293	-	0	0	6.3	-	0	12.5	100	1
18	298	0	50	50	100	100	-	-	-	≥2
19	302	0	0	12.5	50	12.5	100	100	-	≥2
20	304	0	0	12.5	50	6.3	50	100	100	≥2
21	327	0	0	0	25	0	0	12.5	50	2
22	406	d 0	0	0	50	d 0	0	12.5	-	1
23	418	0	0	6.3	12.5	0	0	100	100	1/2
24	444	0	0	0	100	0	6.3	100	100	2
25	622	-	0	0	25	-	25	100	100	≥2
36	983	0	0	0	6.3	0	0	0	100	1/2
45	1030	0	0	0	25	0	0	0	50	1/2
46	1046	0	0	6.3	12.5	12.5	100	100	-	≥2
47	1050	0	6.3	50	100	100	100	100		~1
51	1054	0	0	25	50	50	100	100	-	≥2
56	1059	0	50	100	100	-	100	100	100	≥1/2
57	1062	0	25	100	50	100	100	100	-	≥1/2
59	1064	0	6.3	25	50	100	100	100	-	≥1/2

67	1072		0	0	50	50		50	100	100	-	≥ 2
69	1074		0	0	25	25		25	100	100	-	≥ 2
73	1078		0	0	0	100		0	100	100	100	2
74	1079		0	0	50	100		0	0	-	-	-
77	1094		0	25	50	100		100	100	100	-	≥ 1
78	1096		0	0	25	100		50	100	100	-	≥ 2
80	1098		0	0	0	100		0	100	100	-	2
81	1099		0	0	0	12.5		0	0	100	-	1
82	1100		0	0	6.3	50		0	0	0	6.3	1/4
83	1101		0	0	0	6.3		0	0	0	100	1/2
84	1102		0	0	6.3	50		0	0	100	100	1/2
85	1103		0	0	0	12.5		0	0	12.5	100	1
86	1104	d	0	0	50	100	d	-	0	100	100	1/2
87	1105		0	0	0	50		0	0	12.5	100	1
89	1107	d	0	6.3	50	100	d	-	50	100	100	≥ 1/2
Group B												
90	1		0	25	100	100		0	50	100	100	1/2
91	2		0	0	12.5	50		0	0	50	100	1/2
94	9		0	0	0	25		0	0	0	25	1/2
98	34		0	0	25	200		0	200	200	50	1
100	71		0	0	50	50		0	0	25	100	1/2
101	73		0	0	0	25		0	0	0	25	1/2
107	198		0	0	0	25		0	12.5	50	100	2
Group D												
129	35		0	0	0	25		25	50	50	50	≥ 4
130	36		0	0	0	12.5		25	100	100	100	≥ 4
149	66	c	0	50	50	100	c	0	50	100	100	1/2
151	74	d	0	0	25	100	d	0	25	50	100	1
155	107		0	0	0	100		0	0	0	100	1/2
156	111		0	0	0	0		0	0	0	100	1
158	115		0	0	0	100		0	0	0	50	1/2

160	117		0	0	0	0		0	0	50	100	2
167	129	d	0	0	50	100	d	25	100	200	200	≥ 2
169	149		0	0	25	50		0	0	0	12.5	1/4
172	158	d	0	25	100	100	d	100	200	200	200	≥ 1
178	1120		0	0	12.5	50	b	0	100	100	100	4
179	1121	c	0	0	0	50	c	0	25	50	50	2
183	1157		0	0	0	25		0	0	25	100	1
186	1168	c	0	0	0	25	c	0	100	100	100	2
187	1169	c	0	0	0	50	c	0	100	100	100	2
Group E												
190	52		0	0	25	25	a	0	0	0	25	2
196	92	e	0	0	50	50	e	0	100	100	100	1
198	94	g	0	100	100	100		0	0	50	100	4
199	95	e	0	50	200	200	e	0	100	100	100	1/2
201	99	d	0	25	100	100	d	200	200	200	200	≥ 1/2
209	173	d	0	50	100	200	d	200	200	200	200	≥ 1/2
210	175	c	0	0	12.5	25	c	25	25	50	25	≥ 2
212	178	g	0	0	0	25		0	0	25	50	16
214	182	b	0	0	0	50	b	25	50	100	100	≥ 4
215	184	b	0	0	0	12.5	b	100	100	100	100	≥ 4
218	187	h	0	0	25	100	f	0	0	0	12.5	2
219	189	b	0	0	100	100	b	6.3	6.3	6.3	25	≥ 2
220	192		0	0	0	25		0	0	100	25	2
221	193		0	0	0	12.5		0	0	50	100	1
Group F												
226	58	e	0	0	50	100	e	0	0	50	100	2
237	108	d	0	0	100	100	e	0	100	100	100	1
238	109	d	0	0	25	100	d	0	0	50	50	1/2
239	114	d	0	0	0	100	d	25	50	100	100	≥ 4
251	153	d	0	100	100	100	d	200	200	200	200	≥ 1

256	163	b	0	0	0	12.5	b	25	25	25	50	≧4
258	174	b	0	0	0	50	b	0	12.5	12.5	50	2
259	177		0	0	100	100		0	50	100	100	4
260	179	b	0	0	0	100	b	0	50	25	50	2
261	181	b	0	0	12.5	100	b	25	25	50	100	≧2
262	183	b	0	0	0	25	b	50	50	100	100	≧4
264	190	b	0	0	0	25	b	25	25	100	100	≧4
265	191	f	0	0	0	100	f	50	100	100	50	≧4

Legend : 1) Final concentration γ/cc.

2) Non-toxic concentration. / Inhibitory maximal concentration.

Folloing Letters, a ~ h, in column of Antiviral activity and Cytotoxic activity indicate starting concentrations as follows

a :	800	e :	25
b :	400	f :	12.5
c :	200	g :	6.3
d :	50	h :	3.2

TABLE VI Result of antiviral test of 89 compounds.

Group of compound	Ratio* 1 or less	2	4	16
A	1, 2, 4, 12, 14, 16, 22, 23, 36, 45, 47, 56, 57, 59, 77, 81, 82, 83, 84, 85, 86, 87, 89. Total 23	3, 5, 18, 19, 20, 21, 24, 25, 46, 51, 67, 69, 73, 78, 80. Total 15	11. Total 3	
B	90, 91, 94, 98, 100, 101. Total 6	107. Total 1		
D	149, 150, 155, 156, 158, 169, 172, 173. Total 8	160, 167, 179, 185, 187. Total 5	129, 130, 178. Total 3	
E	196, 199, 201, 209, 221. Total 5	190, 210, 218, 219, 220. Total 5	198, 214, 215. Total 3	212.
F	237, 238, 251. Total 3	226, 258, 260, 261. Total 4	239, 256, 259, 262, 264, 265. Total 6	

Ratio* : Non-toxic concentration / Inhibitory maximal concentration

TABLE VII Summary of antiviral test.

Group of compound	Ratio* 1 or less		2		4		16		Total
	No**	%	No**	%	No**	%	No**	%	
A	23	59	15	38.5	1	25	-	0	39
B	6	86	1	14	-	0	-	0	7
C	-	0	-	0	-	0	-	0	0
D	8	50	5	31.2	3	18.8	-	0	16
E	5	35.7	5	35.7	3	21.4	1	7.2	14
F	3	23	4	30.8	6	46,2	-	0	13
Total	45	50.1	30	33.7	13	14.6	1	1.1	89

Ratio*: Non-toxic concentration / Inhibitory maximal concentration

No** : Numbers of chemical compounds.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of Microbiology, Kitasato University
Tokyo, Japan

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b GROUP

3. REPORT TITLE
STUDIES ON THE ANTIVIRAL ACTIVITY OF GUANYHYDRAZONES ESPECIALLY AGAINST ARBO- AND MYXOVIRUSES (U)

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Final report, 1 March 1965-28 February 1966

5. AUTHOR(S) (Last name, first name, initial)
Nagaki, Daizo

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
1 March 1966	39	9

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
DA-92-557-FEC-37965	J-236
b. PROJECT NO. 2N014501B71D	
c. Task 00 030FE	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. AVAILABILITY/LIMITATION NOTICES
Qualified requesters may obtain copies of this report from DDC.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U. S. Army R&D Group (Far East) APO San Francisco 96343

13. ABSTRACT

Two hundred and sixty-five chemical compounds, guanylhydrazones, azlactones, hydrazones, phenols, carbonyl compounds and miscellaneous ones were tested for in vitro antiviral activity against influenza virus. 104 out of 265 compounds were completely inhibitory by the screening test.

Selected 89 compounds were qualitatively analyzed for the antiviral and toxic activities. 14 out of 89 compounds were found to be inhibitory with the ratio of 4 or more of non-toxic concentration versus minimal inhibitory concentration.

One compound, serial No. 212, synthesis No. 178 inhibited HA production with a final concentration of 1.6γ/cc and was toxic with a final concentration of 25γ/cc. (Author)

DD FORM 1 JAN 64 1473

Unclassified

Security Classification

Unclassified

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Viruses						
Tissue Culture						
Drug Synthesis						
Antiviral Activity						
Hemagglutination Test						
Screening Test						
Toxicity Test						
Japan						

INSTRUCTIONS

1. **ORIGINATING ACTIVITY:** Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. **REPORT SECURITY CLASSIFICATION:** Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. **GROUP:** Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. **REPORT TITLE:** Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. **DESCRIPTIVE NOTES:** If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. **AUTHOR(S):** Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. **REPORT DATE:** Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7a. **TOTAL NUMBER OF PAGES:** The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. **NUMBER OF REFERENCES:** Enter the total number of references cited in the report.

8a. **CONTRACT OR GRANT NUMBER:** If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. **PROJECT NUMBER:** Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a. **ORIGINATOR'S REPORT NUMBER(S):** Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b. **OTHER REPORT NUMBER(S):** If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. **AVAILABILITY/LIMITATION NOTICES:** Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. **SUPPLEMENTARY NOTES:** Use for additional explanatory notes.

12. **SPONSORING MILITARY ACTIVITY:** Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. **ABSTRACT:** Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. **KEY WORDS:** Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.

Unclassified

Security Classification